

Amendments to the Claims:

This listing of claims will replace all previous versions and listings of claims in the application:

1. (Previously presented) A method for amplifying a transcriptionally-active polynucleotide, comprising:

performing a first PCR-amplification step to amplify a first target fragment of DNA with a first primer pair, wherein the first primer pair, upon such amplification, adds to first and second ends of the first fragment predetermined first and second regions of complementarity, to form a second DNA fragment having said first region of complementarity at a first end and a second region of complementarity at a second end of said second DNA fragment;

providing a promoter-containing sequence and a terminator-containing sequence, said promoter-containing sequence further including a region complementary to said first region of complementarity, and said terminator-containing sequence further including a region complementary to said second region of complementarity, wherein both said promoter-containing sequence and said terminator-containing sequence include an internal nucleotide capable of forming an A-T base pair immediately adjacent to said region of complementarity;

joining said promoter-containing sequence to said first end of said second DNA fragment and said terminator-containing sequence to said second end of said second DNA fragment to form said third DNA fragment; and

performing a second PCR-amplification step to amplify said third DNA fragment thereby generating a transcriptionally-active polynucleotide.

2. (Original) The method of claim 1, wherein said joining comprises joining in the presence of polymerase said promoter-containing sequence to said first end of said second DNA fragment and said terminator-containing sequence to said second end of said second DNA

fragment to form said third DNA fragment.

3. (Previously presented) The method of claim 1, wherein said promoter-containing sequence and said terminator-containing sequence further comprise a PNA binding domain.

4. (Canceled)

5. (Previously presented) The method of claim 2, wherein said polymerase is a non blunt end polymerase.

6. (Previously presented) The method of claim 3, wherein the non blunt end polymerase is Taq polymerase.

7. (Previously presented) The method of claim 1, wherein said PCR- amplifying comprises the addition of binding moiety to said third DNA fragment.

8. (Previously presented) The method of claim 7, wherein said binding moiety comprises a PNA molecule.

9.-20. (Canceled)

21. (Currently amended) A method of adding a nucleic acid sequence that confers function to a polynucleotide target sequence, comprising:

contacting first and second nucleic acid fragments with a polynucleotide target sequence, wherein the first nucleic acid fragment comprises a first region of complementarity to a portion of the polynucleotide target sequence and a first extension region, and the second nucleic acid fragment comprises a second region of complementarity to a second portion of the polynucleotide target sequence and a second extension region;

performing a first PCR amplification step comprising amplifying the first and second nucleic acid fragments and the polynucleotide target sequence, to form an intermediate nucleic acid fragment that comprises the polynucleotide target sequence flanked by the first and second extension regions;

contacting the intermediate nucleic acid fragment with third and fourth nucleic acid fragments that respectively comprise a region complementary to the first and second extension regions, wherein ~~one or both of the~~ each of said third and fourth fragments further comprise at least one nucleic acid region that confers function; and

performing a second PCR amplification step comprising amplifying the intermediate nucleic acid fragment with the third and fourth nucleic acid fragments to form a product nucleic acid fragment that comprises ~~one or more than one~~ functional nucleic acid regions joined to the polynucleotide target sequence.

22. (Currently amended) The method of claim 21, wherein the product nucleic acid fragment comprise a promoter sequence ~~or-a~~ and terminator sequence ~~or-both~~.

23. (Previously presented) The method of claim 21, wherein said product nucleic acid fragment further comprises a PNA binding domain.

24. (Previously presented) The method of claim 21, wherein said PCR amplifying of the intermediate nucleic acid fragment further comprises a set of primers, wherein at least one primer comprises a nuclease resistant, binding moiety that upon amplification confers nuclease resistance to said product nucleic acid fragment.

25. (Currently amended) The method of claim 24, wherein said one or more nuclease resistant, binding moieties comprise a PNA molecule ~~or-one or more phosphorothioate molecules or-both~~.

26. (Canceled)

27. (Previously presented) The method of claim 21, wherein said PCR amplifying steps are accomplished using a polymerase, and wherein said polymerase is a non blunt end polymerase.

28. (Previously presented) The method of claim 27, wherein said non blunt end polymerase is Taq polymerase.

29. (Previously presented) The method of claim 21, wherein said nucleic acid region that confers function comprises a promoter or a terminator.

30. (Previously presented) A system for adding a nucleic acid fragment that confers function to a polynucleotide target sequence, comprising:

an extension primer pair comprising a 5' and a 3' primer, wherein the 5' primer comprises a region of complementarity to a 5' strand of the polynucleotide target sequence and a predetermined extension region, and wherein the 3' primer comprises a region of complementarity to a 3' strand of the polynucleotide target sequence and a predetermined extension region; and

a 5' biological function conferring nucleic acid fragment and a 3' biological function conferring nucleic acid fragment, each fragment of which comprises a region of complementarity to one of the extension regions, and a biological function conferring polynucleotide sequence that confers biological function, wherein the extension primer pairs are adapted to add the extension regions to a target sequence upon a first PCR procedure, and the function conferring nucleic acid pairs are adapted to add the functional polynucleotide sequences to the polynucleotide target sequence upon a second PCR procedure.

31. (Previously presented) The system of claim 30, wherein the system further comprises a polymerase.

32. (Previously presented) The system of claim 31, wherein the polymerase is a non blunt end polymerase.

33. (Previously presented) The system of claim 32, wherein the non blunt end polymerase is Taq polymerase.

34. (Previously presented) The system of claim 30, wherein the 5' biological function conferring nucleic acid fragments comprises a promoter.

35. (Previously presented) The system of claim 34, wherein the biological function conferring nucleic acid fragments further comprise at least one PNA binding domain.

36. (Previously presented) The system of claim 30, further comprising an additional primer pair comprising at least one nuclease-resistant, binding moiety.

37. (Currently amended) The system of claim 36, wherein the nuclease-resistant binding moiety ~~is selected from the group consisting of~~ comprises a PNA molecule and a phosphorothioate.

38. (Currently amended) A method for creating transcriptionally-active nucleic acid sequences from a plurality of different target polypeptide-encoding DNA sequences, comprising:

creating extension primer pairs for each of a plurality of different target polypeptide-encoding sequences, each extension primer pair comprising first and second extension primers, respectively comprising first and second extension regions and a region of complementarity to a particular target sequence, such that the first and second extension regions for each extension primer pair are the same as the first and second extension regions for the other of said extension primer pairs, but the regions of complementarity are customized for each target sequence;

performing a first PCR amplification step comprising amplifying each of said target sequences with said extension primer pairs to provide intermediate sequences comprising said plurality of target sequences, each said target sequence flanked by the same first and second extension regions; and

providing transcriptionally-functional fragment pairs, wherein each transcriptionally-functional fragment pair comprises a first fragment having a region of complementarity to the first extension region and a second primer having a region of complementarity to the second extension region, and ~~at least one of~~ each of said fragments in said transcriptionally-functional fragment pair ~~comprising~~ comprises a transcriptionally-functional region; and

performing a second PCR amplification step comprising amplifying each of said intermediate sequences with said transcriptionally-functional fragment pairs to provide a plurality of transcriptionally-functional polynucleotides, each comprising one of the target sequences linked to at least one transcriptionally-functional regions.

39. (Currently amended) The method of claim 38, wherein one of said transcriptionally-functional regions, is a promoter and one of said transcriptionally-functional regions is [[or]] a terminator sequence.

40. (Canceled)

41. (Previously presented) The method of claim 38, wherein the PCR amplifications are performed separately for each of said target sequences.

42. (Canceled)

43. (Previously presented) The method of claim 38, wherein said PCR amplifying steps are accomplished using a polymerase, and wherein said polymerase is a non blunt end polymerase,

44. (Currently amended) A method of generating a nuclease resistant nucleic acid molecule, comprising:

contacting first and second nucleic acid fragments with a polynucleotide target sequence, wherein the first nucleic acid fragment comprises a first region of complementarity to a portion of the polynucleotide target sequence and a first extension region, and the second nucleic acid fragment comprises a second region of complementarity to a second portion of the polynucleotide target sequence and a second extension region;

performing a first PCR amplification step comprising amplifying the first and second nucleic acid fragments and the polynucleotide target sequence, to form an

intermediate nucleic acid fragment that comprises the polynucleotide target sequence flanked by the first and second extension regions;

contacting the intermediate nucleic acid fragment with a third and a fourth nucleic acid fragments that respectively comprise a region complementary to the first and second extension regions and with a first primer and a second primer at least one of which comprises a nuclease-resistant, binding moiety, wherein ~~one or both~~ each of the third and fourth nucleic acid fragments further comprise a nucleic acid region that confers function; and

performing a second PCR amplification step comprising amplifying the intermediate nucleic acid fragment with the third and fourth nucleic acid fragments and the first and second primers to form a nuclease resistant nucleic acid molecule that comprises ~~one or more~~ first and second functional nucleic acid regions joined to the polynucleotide target sequence, and at least one nuclease-resistant, binding moiety on a 5' end.

45. (Currently amended) The method of claim 44, wherein, said nuclease-resistant, binding moiety [[is]] comprises a PNA molecule ~~and/or one or more phosphorothioate molecules~~.

46. (Previously presented) The system of claim 30, wherein the 3' biological function conferring nucleic acid fragments comprises a terminator.